

Rapid Molecular Detection of ESBL gene variants with a novel Ligation-mediated Real Time PCR

R.H.T. Nijhuis¹#, P.H.M. Savelkoul², A.A. van Zwet¹

Introduction

Extended spectrum beta-lactamases (ESBLs) are emerging in both the community and in hospitals and have increasingly been described worldwide since their introduction, with bla_{TEM}, bla_{SHV} and CTX-M being the most frequently detected and clinically important ESBLs.

Because of the emergence of ESBLs, rapid and adequate detection of ESBLs is crucial for both infection control measures as well as for the choice of correct antimicrobial therapy.

Methods

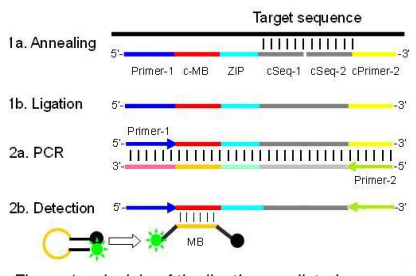


Figure 1: principle of the ligation-mediated real time PCR

- From June - October 2011, all ESBL positive strains from clinical specimens based on Vitek2 results were included in the study
- Strains were tested with the phenotypic Combined Disc Test (CDT) and the ligation mediated Real Time PCR (LM-RT PCR) (Check-Points, Wageningen, The Netherlands) as illustrated in figure 2
- Discrepant results comparing LM-RT PCR and CDT were tested using the Check-MDR CT103 assay (Check-Points, Wageningen, The Netherlands), which is able to detect most prevalent ESBLs, pAmpCs and carbapenemases.

- A subset of these isolates was used to compare the TAT

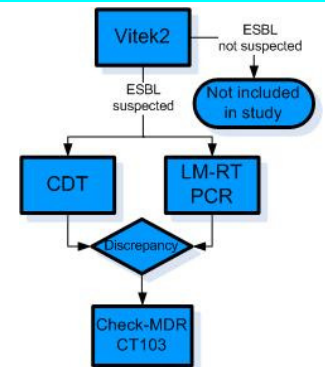


Figure 2: flowchart study

Results (performance)

➤ Of 195 putative ESBL producing isolates, 106 (54.4%) and 95 (48.7%) obtained positive results using CDT and LM-RT PCR respectively

➤ Comparing CDT and LM-RT PCR, 13 discrepancies were found (table 1)

➤ Discrepancy testing using the Check-MDR CT103 assay confirmed all LM-RT PCR results (table 2)

Table 1: performance LM-RT PCR

	LM-PCR+	LM-PCR-
CDT+	94	12
CDT-	1	88
Sensitivity	98.9%	
Specificity	88%	
PPV	88.7%	
NPV	98.9%	

Table 2: results discrepancy testing

	CDT	LM-RT PCR	Check-MDR CT103
<i>Achromobacter</i> spp	+ (1)	- (1)	No signal* (1)
<i>Acinetobacter</i> spp	+ (4)	- (4)	No signal* (4)
<i>E. cloacae</i>	+ (2)	- (2)	AmpC positive (2)
<i>E. coli</i>	+ (4)	- (4)	No signal* (1) WT-TEM + AmpC (1) WT-TEM (2)
	- (1)	+ (1)	CTX-M1 ESBL (1)
<i>S. multivorum</i>	+ (1)	- (1)	Indeterminate (1)

Results (TAT)

➤ Of 63 isolates, 38.1% could gain results the same day and the remaining 61.9% the next day using LM-RT PCR, whereas CDT gained 96.8% of the results the next day

➤ TAT varied from 5:25hrs – 24:50hrs and 16:45hrs – 49:25hrs (mean: 15:20hrs and 24:15hrs) for the LM-RT PCR and CDT respectively

➤ In >90% of all isolates included, TAT of LM-RT PCR was shorter than the CDT (figure 3)

Conclusion

The ligation-mediated real time PCR provides an important reduction in turn-around-time for ESBL confirmation. As a consequence, all ESBL results are available within the same day, making this assay an important tool for rapid and accurate ESBL detection.

Comparison turn-around-time

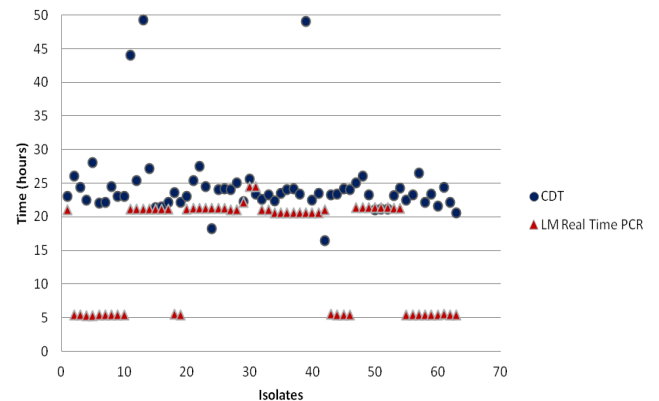


Figure 3: comparison turn-around-time

1. Rijnstate
Dept. of Med. Microbiology and Immunology
President Kennedylaan 100
6883 AZ VELP
The Netherlands
Phone: +31 88 0055455
Fax: +31 88 0055755

2. VU University Medical Center
Dept. of Med. Microbiology and Infection control
De Boelelaan 1118
1081 HV AMSTERDAM
The Netherlands